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# Postharvest exogenous melatonin treatment of strawberry reduces postharvest spoilage but affects components of the aroma profile

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## ABSTRACT

**Background:** Strawberries are perishable fruits that decay quickly after harvest, but are valued for their distinctive taste and aroma. Melatonin is involved in plant resistance against stress, plant senescence and fruit ripening, and was shown to delay post-harvest spoilage of strawberries.

**Objective:** The effects of melatonin postharvest treatment on shelf-life and volatile organic compound profile were assessed in strawberry fruits cv “Luca”.

**Methods:** Strawberry fruit were treated with 100 µM melatonin and stored at 4 °C for 12 days to assess whether melatonin treatment could delay spoilage without adversely affecting aroma.

**Results:** Melatonin treatment delayed fruit deterioration by reducing weight loss and incidence of decay as well as maintaining total soluble solids, titratable acidity, anthocyanin, and taste. Melatonin treatment also significantly reduced CO<sub>2</sub> production compared to control fruits. The relative abundance of the majority of volatile organic compounds (VOCs) was not affected, however abundance of two VOCs that are important components of strawberry aroma were affected by melatonin treatment.

35 **Conclusions:** Post-harvest treatment of strawberries with 100  $\mu$ M melatonin improved strawberry  
36 quality and conserved bioactive compounds after 12 d of storage. However, components of the aroma  
37 profile were altered in a way which may affect consumer perception of quality.

38

39 **Key words:** ethyl hexanoate, *Fragaria*×*ananassa*, melatonin, postharvest quality, volatile organic  
40 compounds.

## 41 **1. Introduction**

42 North and West African countries are considered as the main suppliers of fresh fruits and vegetables  
43 to northern Europe and most EU countries [1]. This supply chain requires long periods of transport  
44 and storage under controlled refrigerated conditions, which can result in the proliferation of  
45 postharvest spoilage microbiota [2], as well as fruit deterioration through bruising. Two major  
46 problems are linked with the globalised supply chain for fruit and vegetables: postharvest loss of  
47 produce and the dispersal of human pathogens with produce.

48 Strawberries are a high value crop, rich in bioactive compounds of known health benefit. These  
49 include vitamins, especially vitamin C and E, and other bioactive compounds such as  $\beta$ -carotene and  
50 phenolic compounds [3]. However, as is the case with all soft fruit, strawberry shelf life is very  
51 limited, leading to substantial waste in the supply chain [4]. Strawberries are considered as one of the  
52 most non-climacteric perishable fruits, with a very limited shelf life [5]. There is therefore a need for  
53 a treatment which stabilises strawberry quality after harvest and prolongs shelf life.

54 Melatonin is produced by all eukaryotes including strawberry fruit [6] and is a potent antioxidant.  
55 Moreover, exogenous treatment of plants with melatonin can mitigate effects of abiotic stress by  
56 reducing reactive oxygen species and interacting with hormone signalling [7]. Melatonin can also be  
57 applied to fruit post-harvest. Exogenous postharvest melatonin application at 0.1 or 1.0 mmol L<sup>-1</sup> to  
58 strawberry cv. Hongyan fruit delayed fruit senescence, reduced decay and weight loss, while total  
59 phenolics increased, resulting in higher antioxidant content [8]. Similarly, exogenous 100  $\mu$ mol L<sup>-1</sup>  
60 melatonin treatment resulted in a reduction in decay and increased total phenolic compounds and

anthocyanins in strawberry cv. Selva fruits [9]. However, little is known about the effects of melatonin on VOCs, key components of the aroma. An alteration in flavour-related VOCs would have implications for the use of melatonin as a treatment to improve quality and postharvest shelf-life of the fruit. We hypothesised that the effects of melatonin treatment might affect the profile of volatile compounds.

## 2. Materials and methods

### 2.1. Plant material, melatonin treatments and storage conditions

Strawberry fruit (*Fragaria x ananassa* Duch., cv. Luca) were collected at the commercial maturity stage (98 % red colour, assessed visually, and 4.5 week after flowering) from a local farm (Haygrove, Cardiff). The fruits were immediately transported to Cardiff University, School of Biosciences, Wales, UK. Fruit were then carefully sorted to exclude misshapen, overripe, underripe or damaged fruit. The experiment began on the same day. The sorted fruits were randomly divided into three homogenous groups of 300g, representing the number of treatments. Strawberries were immersed in three different solutions for 5 min: control (distilled water), 50  $\mu$ M or 100  $\mu$ M melatonin in distilled water. Following immersion, the fruits were dried for 2 hours at room temperature (RT) in a sterile flow hood. The fruits were placed in polyethylene trays (clamshells), then stored for 12 days at 4 °C and 90 % relative humidity. The experiment was repeated twice, with three replicates for each treatment in each of the two experiments. Since no significant differences between 0 and 50  $\mu$ M concentrations of melatonin were found in any of the parameters tested, results are only presented for the control and 100  $\mu$ M melatonin treatments.

### 2.2. Weight loss and decay assessments

87 To determine weight loss, strawberry fruits were weighed immediately after air-drying and at every  
88 sampling time. The results are shown as the percentage weight loss compared to the initial fresh  
89 weight.

90 For evaluation of decay, decay percentage (%) was calculated by weighing all decayed fruits (affected  
91 by visible signs of rot or fungal growth) relative to the weight of total fruits. Fruits free from any  
92 decay were used for further analyses. The fruits were sliced in small pieces and immersed  
93 immediately in liquid nitrogen and stored at -80 °C until used to measure titratable acidity (TA),  
94 anthocyanin and phenolic content.

95

### 96 *2.3. Analysis of colour, total soluble solids (TSS), pH, and titratable acidity (TA)*

97

98 The colour of the strawberry surface was measured with a digital camera (Cannon, Japan). Six fruits  
99 per replicate were used for the colour test. Images were calibrated using Image J software.

100 TSS in strawberry juice was measured using a refractometer (RHB-18ATC). The TSS reading (in  
101 degree Brix, Bx) was expressed as the % of TSS in the fruit.

102 To measure TA, three fruits from every replicate were homogenized with a tissue homogenizer for 5  
103 min then 5 g of strawberry fruit juice was diluted to 50 mL with distilled water and titrated to pH 8.1  
104 using 0.1 M NaOH. TA was calculated according to AOAC [10] and expressed as percentage of citric  
105 acid equivalent, since citric acid is the principal acid in strawberries [11]. The pH of the juiced  
106 strawberry fruit was measured with a calibrated pH meter (EuTech, Instruments, pH 510, Singapore).

107

### 108 *2.4. Taste index*

109

110 Taste index (TI) was calculated with the equation below [12] using the TSS and TA values:

111 
$$TI = (TSS \text{ value}/20 \times TA \text{ value}) + TA \text{ value}$$

112

113 2.5. *CO<sub>2</sub>/O<sub>2</sub> emission rate and ethylene production rates*

114

115 To measure the CO<sub>2</sub>/O<sub>2</sub> emission rate, individual strawberry fruits were placed in 250 mL sealed glass  
116 containers and after 1 h of enclosure at room temperature (20 °C), 1mL of air sample was extracted  
117 from the headspace and analyzed using a gas analyser (Model ML206, AD-Instruments, Australia)  
118 for CO<sub>2</sub>/O<sub>2</sub> ratio.

119 To measure ethylene production, strawberry fruits were incubated in a 250 mL super sealed glass jar  
120 for 60 min at room temperature (25 °C). Each strawberry fruit sample was weighed using an analytical  
121 balance to record its initial weight. The caps of the containers were drilled (1 mm in diameter), to  
122 enable head space gas sampling. A rubber seal was inserted to cover the hole to prevent the gas inside  
123 the container leaking. One mL of the headspace was sampled from the glass jar via an air tight syringe  
124 (SGE, Analytical Science, PA, USA), and injected immediately into a gas chromatograph (Agilent  
125 Technology, 6890N GC system, USA) fitted with an Alumina Sulfate Plot column (30 m x 0.32 mm,  
126 Supelco) column and a FID detector. Column temperature was 70 °C and injection temperature was  
127 120 °C. Helium was used as a carrier gas with a flow rate of 1 mL min<sup>-1</sup>. The rate of ethylene emission  
128 was expressed as  $\mu\text{l C}_2\text{H}_4 \text{ kg}^{-1} \text{ FW h}^{-1}$  using ChemStation software (Rev.A.09.01, Agilent  
129 technologies, USA).

130

131 2.6. *Collection and analysis of Volatile organic compounds (VOCs)*

132

133 At day 0 and 4 of cold storage at 4 °C, the lids of the containers were removed, the samples were  
134 sealed into a 25 cm x 38 cm nalophene plastic bag (TJM Ltd), and they were equilibrated for 2 hours  
135 at room temperature (20 °C). Headspace gas (400 mL) was collected using a hand pump (Easy VOC  
136 pump, Markes International Ltd.) onto thermal desorption tubes packed with Tenax TA and SulfiCarb  
137 sorbents (Markes International Ltd.).

138 The VOCs collected for both experiments were then analysed after thermal desorption by gas  
139 chromatography and time of flight mass spectrometry (TD-GC-TOF-MS) essentially as described by  
140 [Spadafora et al. \[13\]](#). A TD100 (Markes International Ltd.) was used to desorb tubes and inject  
141 samples into the GC. Samples were desorbed onto the trap (at 25 °C) first for 5 min at 120 °C and  
142 then for 5 min at 260 °C with 40 mL min<sup>-1</sup> nitrogen. The trap was desorbed at 300 °C for 3 min with  
143 40 mL min<sup>-1</sup> helium resulting in a split ratio of 20:1 into the GC (7890A, Agilent Technologies, Inc.).  
144 VOCs were separated on a 60 m, 0.32 mm I.D. and 0.5 µm film thickness Rxi-5ms capillary column  
145 (Restek). The temperature program was: 40 °C for 5 min, 10 °C min<sup>-1</sup> ramp to 300 °C, final hold 5  
146 min (total run time 41 mins). A BenchTOF-dx MS (Almsco International) was used to detect VOCs.  
147 It was operated at a source temperature of 275 °C and filament voltage of 1.6 V. Ions were collected  
148 in the mass range 35-500 m/z. A retention time standard (C8-C20, Sigma Aldrich) was run with each  
149 set of samples and prepared by injecting 1 µl of the standard mixture directly onto a TD tube (Tenax  
150 TA).  
151 Data was processed and analysed using AMDIS (NIST 2014) and MSD ChemStation software  
152 (E.02.01.1177, Agilent Technologies, Inc.). A custom MS library was produced using retention  
153 indices (MS spectra were searched against the NIST library with over 80 % identification in forward  
154 and backward fit). Putative identification was made on the basis of >80 % match of mass spectra and  
155 +/-15 in retention index to the custom library. These were then processed to remove contaminants,  
156 defined as compounds not present in at least two replicates, and compounds present at similar levels  
157 in control samples.

158

## 159 *2.7. Total anthocyanin content*

160

161 Total anthocyanin was measured according to previously published methodology [[14](#)]. Fruit pulp (2  
162 g) was blended with extraction solvent (20 mL of ethanol, 1.5 N HCl, 85: 15) and kept overnight at  
163 4 °C. The samples were then filtered into a volumetric flask and covered with aluminium foil. The



164 remaining residue was washed with extraction solvent until the pigments were removed. Filtrates  
165 were pooled and made up to 100 mL with extraction solvent. Absorbance was recorded at 535 nm to  
166 determine the anthocyanin content using the following formula: Absorbance at 535 nm  $\times$  volume of  
167 extraction solution  $\times$  100/ weight of sample  $\times$  98.2. The results are expressed as mg/100 g fresh  
168 weight.

169

## 170 2.8. Total phenolic content

171

172 Strawberry fruits from each treatment were homogenized using a laboratory blender. The mixture  
173 was centrifuged at 5000 g for 20 min at room temperature. The supernatant was filtered through a  
174 paper filter (Whatman N. 1) to yield a clear juice. Total soluble phenolics were measured using Folin–  
175 Ciocalteu reagent according to published methodology [15]. Briefly, 0.5 mL juice aliquots were  
176 diluted in 9.5 mL distilled water. Then, to 1 mL of the resulting solution, 5 mL of a diluted (1 + 9  
177 distilled water) Folin–Ciocalteu reagent (Fisher Scientific International, Leicestershire, UK) were  
178 added. Then 4 mL of sodium carbonate solution 7.5 % (BDH Limited, Poole, England) were added  
179 and after 1 h at 30° C and 1 h at 0° C, the absorbance of the solution was measured at 760 nm with a  
180 model SP8-400 UV/VIS Spectrometer (Pye, Unicam Ltd, Cambridge, England). Using gallic acid  
181 (97-5.102.5 % (titration) Sigma Aldrich, China) as the standard. Results are expressed as mg of gallic  
182 acid equivalents (GAE) per L.

183

## 184 2.9. Statistical analysis

185

186 All physiological and biochemical data were analysed statistically using a one-way ANOVA test,  
187 where  $P$ -values  $< 0.05$  were considered significant, using SPSS 19 statistical software. The mean  
188 values  $\pm$  SE were compared using a Tukey test. Changes in individual VOC abundance at each time  
189 point were analysed using a Student's  $t$ -test. Analysis of total VOC profiles was performed essentially



190 as described in Spadafora et al. [13]. Peak areas were normalised to the total area of the chromatogram  
191 for each sample, and the square root of the area was used for further analysis to reduce the contribution  
192 of larger component areas. Using the R platform (version 3.1.3; R core development team 2015) data  
193 were analysed using PerMANOVA (Permutational Multivariate Analysis of Variance) and CAP  
194 analysis (Canonical Analysis of Principal coordinates) tests [16]. These were carried out using the  
195 ‘vegan’ and BiodiversityR’ packages within R. This analysis treats the whole profile as a single  
196 independent variable. An ordination plot was generated by the software and a 95% confidence interval  
197 was fitted to the data.

198

### 199 **3. Results and Discussion**

200

#### 201 *3.1 Weight loss and decay are significantly affected by treatment with melatonin*

202

203 Melatonin treated fruits lost significantly ( $P < 0.05$ ) less weight than the controls starting from 2 days  
204 of storage, with the difference increasing until the end of the storage period (Figure 1A). Moreover,  
205 overall weight loss decreased significantly by 59.76 % in melatonin treated compared with control  
206 fruits. There was no decay observed until day 4 of storage (Figure 1.B; Supplementary Table 1). After  
207 this, decay was reduced in the melatonin treated fruits both at days 8 and 12 of storage, and by 50.01%  
208 compared to the controls at 12 days of storage. Similar effects of melatonin were shown previously  
209 on decay in strawberry<sup>11</sup> and on both decay and weight loss in peach [17]. The reduced decay of the  
210 strawberry fruits elicited by the 100  $\mu$ M melatonin treatment could be due to its effects on reactive  
211 oxygen species resulting in increased cell wall rigidity. Higher superoxide dismutase enzyme activity  
212 in parallel with lower ascorbate peroxidase and catalase enzyme activity in fruits was shown to result  
213 in H<sub>2</sub>O<sub>2</sub> accumulation as a result of melatonin treatment of strawberry fruit [9]. This in turn activated  
214 enzymes in the phenylpropanoid pathway, which enhance cell wall rigidity and the nutritional quality  
215 of the fruit. Calcium also plays an important role in cell wall structure and tissue firmness. Melatonin

216 application led to a reduction in water loss and an increase in calcium content in maize seedlings  
217 under cold stress [18]. The weight loss and reduced decay elicited by melatonin treatment in chilled  
218 strawberry fruit shown here could therefore possibly be due to redistribution of the calcium in  
219 strawberry fruit tissue leading to less moisture loss. Post-harvest dipping in calcium solutions and  
220 thus increasing calcium content is known to decrease the decay of strawberry fruits [19].

221

222 *3.2 Taste index was improved by melatonin treatment: while total soluble sugars increased, titratable*  
223 *acidity was unaffected*

224

225 Total soluble solids content (TSS) increased slightly but significantly ( $P < 0.05$ ) from 0 to 2-4 days  
226 of storage and decreased thereafter in both treatments (Supplementary Table 1). Fruits treated with  
227 100  $\mu\text{M}$  melatonin had a significantly higher TSS than the control after 4 and 8 days of storage,  
228 whereas at the last storage time point, there was no significant difference between treatments. Our  
229 results are in accordance with Gao et al. [17] who reported that melatonin treatment significantly  
230 increased retention of TSS content during cold storage of peach fruits. In addition, tomato plants  
231 supplemented with melatonin showed significant increases in their contents of TSS in fruits [20].

232 There was no significant difference in TA between 100  $\mu\text{M}$  melatonin treated and control strawberry  
233 fruits until 4 days of storage (Figure 2A). However, after 8 and 12 days of storage, TA was  
234 significantly higher in the melatonin treated fruits than in the controls. Previous work [20] reported  
235 that tomato plants irrigated continuously with melatonin had significantly higher content of citric acid  
236 in fruits. They suggested that organic acids were enhanced by melatonin treatment. The pH of  
237 strawberry fruits was significantly higher in the control than in the treated fruits at the end of the  
238 storage period (Figure 2.B), however, there was no significant difference between the two treatments  
239 at other time points.

240 The taste index (%) of melatonin treated fruits was significantly higher ( $P < 0.05$ ) than control fruits  
241 at 8 and 12 days of storage (Figure 2C and Supplementary Table 1). The higher taste development

induced by melatonin treatment might be due to a general increase in total sugars and organic acids [21].

### *3.3 Treatment with melatonin affected anthocyanin and phenolic content at selected storage time points*

Anthocyanin increased significantly ( $P < 0.05$ ) with storage time (Supplementary Table 1). The treatment with melatonin increased anthocyanin content in the fruits compared to the control from 4 days until 8 days of storage, while there was no significant difference at the end of the storage period. Previously, Aghdam and Fard [9] found higher anthocyanin content in strawberry fruits treated with melatonin compared to the control. They proposed that this may be due to the higher phenylalanine ammonia lyase (PAL) enzyme activity which leads to accumulation of anthocyanins.

Total Phenols decreased up to 4 days of cold storage then slightly increased ( $P < 0.05$ ) Figure 3B and Supplementary Table 1). Total phenols were significantly higher ( $P < 0.05$ ) in fruits treated with melatonin than in control fruits at 2 and 4 days of storage, however there were no differences at 8 and 12 days between treatments. Similar results were previously reported in strawberry [8] indicating that total phenol content is significantly increased by melatonin treatment in strawberry fruit. The increase in total phenol contents in strawberry fruit might be explained by a higher phenylalanine ammonia lyase (PAL) enzyme activity induced by the melatonin treatment leading to an accumulation of phenols [9].

### *3.4 CO<sub>2</sub> production increased with melatonin treatment while ethylene production and the overall profile of volatile organic compounds was unaffected*

CO<sub>2</sub> production rate, which is considered here as a proxy for the respiration rate, was significantly higher in control fruit than melatonin treated fruits throughout storage until the last time point (Figure

3C). However, there were no statistically significant differences observed in ethylene production (Supplementary Table 2). Strawberry fruit is considered non-climacteric and produces a low level of ethylene [22] which is consistent with the results seen here. However, the relationship between respiration rate and melatonin treatment is unclear: the increase in respiration seen in the melatonin treated fruit could be due to the role of melatonin in plant metabolism and an alteration to the balance of plant growth regulators [23] however further studies are needed.

Fifty volatile organic compounds (VOCs) were identified from all the samples including esters (total 33 with eight acetate and 25 non-acetate), aromatic compounds (5), alkanes (3), furans (3), ketones (2), terpenes (2) and nitriles (1) (Supplementary Table 3). Analysis using PerMANOVA indicated that the overall VOC profile changed across time  $P < 0.001$ ,  $R^2 = 0.470$ ). Linear discrimination plots based on CAP analysis separated the two storage time points with 100% correct classification (Figure 4). However, there was no discrimination between melatonin treated and non-treated fruits (Supplementary Table 4). A closer examination of changes in individual VOCs in treated vs. control fruit at the two time points reveals that indeed the majority of VOCs did not change in abundance significantly. However ethyl 2-methylbutanoate was absent from fruit treated with melatonin at day 0 while it was present in the control fruit, and ethyl hexanoate was 2-fold more abundant in the headspace of control fruit after 4 days of storage compared to fruit treated with melatonin ( $P < 0.05$ ; Table 1; Supplementary Table 4). Both VOCs have been reported as important components of strawberry bouquets previously [24], however their abundance varied across different cultivars and abundance of ethyl hexanoate also increased with storage. Further sensorial analysis would be required to assess whether the changes noted here in VOC profile following melatonin treatment significantly change the perceived aroma of the fruit.

290

#### 291 4. Conclusion

292

293 We confirmed previous work showing that exogenous treatment of strawberry fruit with melatonin  
294 at 100  $\mu$ M would delay fruit deterioration. In fact, it decreased weight loss, decay and respiration rate  
295 during cold storage at 4° C. In addition, the treatment increased TSS, anthocyanin, and total phenols  
296 compared to the control. However, melatonin treatment did not affect ethylene production or colour  
297 intensity. Although overall volatile organic compound profile was not affected by the melatonin  
298 treatment, the abundance two important aroma related VOCs was affected. The quality of strawberry  
299 could therefore be enhanced by treatment of melatonin however the changes to the aroma may affect  
300 consumer quality assessments of the fruit. Further studies are needed to fully understand the  
301 mechanism of melatonin action on non-climacteric fruits, and the organoleptic effects on fruit aroma.

302

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307

### 308 **Compliance with ethical standards**

### 309 **Conflict of interest statement**

310 The authors declare no conflict of interest.

### 311 **Compliance with ethics requirements**

312 This article does not contain any studies with human or animal subjects.

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### 319 **References**

320 [1] (<https://www.cbi.eu>).

- 321 [2] El-Mogy MM, Alsanius BW. Cassia oil for controlling plant and human pathogens on fresh  
322 strawberries. Food Control. 2012; 28: 157-162.DOI: 10.1016/j.foodcont.2012.04.036
- 323 [3] Manganaris GA, Goulas V, Vicente AR, Terry LA. Berry antioxidants: small fruits providing  
324 large benefits. Journal of the Science of Food and Agriculture. 2014; 94: 825–833.DOI:  
325 10.1002/jsfa.6432.
- 326 [4] Nunes MCN, Brecht JK, Sargent SA, Morais AMMB. Controlling temperature and water loss to  
327 maintain ascorbic acid levels in strawberries during postharvest handling. Journal of Food  
328 Science. 1998; 63: 1033–1036. DOI: 10.1111/j.1365-2621.1998.tb15848.x.
- 329 [5] Ayub RA, Reis L, Bosetto L, Lopes PZ, Galvão CW, Etto RM. Brassinosteroid plays a role on  
330 pink stage for receptor and transcription factors involved in strawberry fruit ripening. Plant  
331 Growth Regulation. 2017; 84: 159–167. DOI: 10.1007/s10725-017-0329-5.
- 332 [6] Stürtz M, Cerezo AB, Cantos-Villar E, Garcia-Parrilla MC. Determination of the melatonin  
333 contents of different varieties of tomatoes (*Lycopersicon esculentum*) and strawberries  
334 (*Fragaria ananassa*). Food Chemistry. 2011; 127:1329–1334. DOI:  
335 10.1016/j.foodchem.2011.01.093.
- 336 [7] Sun Q, Zhang N, Wang J, Zhang H, Li H, Shi J, Li R, Weeda S, Zhaol B, Ren S, Guo Y.  
337 Melatonin promotes ripening and improves quality of tomato fruit during postharvest  
338 life. Journal of Experimental Botany. 2015; 66: 657–668. DOI: 10.1093/jxb/eru332.
- 339 [8] Liu C, Zheng H, Sheng K, Liua W, Zheng LI. Effects of melatonin treatment on the postharvest  
340 quality of strawberry fruit. Postharvest Biology and Technology. 2018; 139: 47–55. DOI:  
341 10.1016/j.postharvbio.2018.01.016.
- 342 [9] Aghdam MS, Fard JR. Melatonin treatment attenuates postharvest decay and maintains nutritional  
343 quality of strawberry fruits (*Fragaria x anannasa* cv. Selva) by enhancing GABA shunt  
344 activity. Food Chemistry. 2017; 221: 1650–1657. DOI: 10.1016/j.foodchem.2016.10.123.
- 345 [10] AOAC (1990) Titratable Acidity 942.15, 15th ed. AOAC Official Methods of Analysis.

- 346 [11] Watson R, Wright CJ, McBurney T, Taylor AJ, Linforth RST. Influence of harvest date and  
347 light integral on the development of strawberry flavour compounds. *Journal of Experimental*  
348 *Botany*. 2002; 53: 2121–2129. DOI: [org/10.1093/jxb/erf088](https://doi.org/10.1093/jxb/erf088).
- 349 [12] Pezzarossa B, Rosellini I, Borghesi E, Tonutti P, Malorgio F. Effects of Se-enrichment on yield,  
350 fruit composition and ripening of tomato (*Solanum lycopersicum*) plants grown in  
351 hydroponics. *Scientia Horticulturae*. 2014; 165: 106-110. DOI:  
352 [10.1016/j.scienta.2013.10.029](https://doi.org/10.1016/j.scienta.2013.10.029).
- 353 [13] Spadafora ND, Amaro AL, Pereira MJ, Müller CT, Pintado M, Rogers HJ. Multi-trait analysis  
354 of post-harvest storage in rocket salad (*Diplotaxis tenuifolia*) links sensorial, volatile and  
355 nutritional data. *Food Chemistry*. 2016; 211:114–123. DOI:  
356 [org/10.1016/j.foodchem.2016.04.107](https://doi.org/10.1016/j.foodchem.2016.04.107).
- 357 [14] Lees DH, Francis FJ. Standardization of pigment analyses in cranberries. *HortScience*. 1972; 7:  
358 83-84.
- 359 [15] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic  
360 acid reagents. *American Journal of Enology and Viticulture*. 1965; 16: 144–157.
- 361 [16] Anderson MJ, Willis TJ. Canonical analysis of principal coordinates: a useful method of  
362 constrained ordination for ecology. *Ecology*. 2003; 84: 511-525.
- 363 [17] Gao H, Zhang ZK, Chai HK, Cheng N, Yang N, Wang DN, Yang T, Cao W. Melatonin treatment  
364 delays postharvest senescence and regulates reactive oxygen species metabolism in peach  
365 fruit. *Postharvest Biology and Technology*. 2016; 118:103–110. DOI:  
366 [org/10.1016/j.postharvbio.2016.03.006](https://doi.org/10.1016/j.postharvbio.2016.03.006).
- 367 [18] Turk H, Erdal S. Melatonin alleviates cold-induced oxidative damage in maize seedlings by up-  
368 regulating mineral elements and enhancing antioxidant activity. *Journal of Plant Nutrition*  
369 *and Soil Science*. 2015; 178: 433-439. DOI: [10.1002/jpln.201400476](https://doi.org/10.1002/jpln.201400476) 433.
- 370 [19] García J M, Herrera S, Morilla A. Effects of Postharvest Dips in Calcium Chloride on  
371 Strawberry. *Journal of Agriculture and Food Chemistry*. 1996; 44: 30–33. DOI:



372 10.1021/jf950334l.

373 [20] Liu J, Zhang R, Sun Y, Liu Z, Jin W and Sun Y. The beneficial effects of exogenous melatonin  
374 on tomato fruit properties. *Scientia Horticulturae*. 2016; 207: 14–20. DOI:  
375 10.1016/j.scienta.2016.05.003.

376 [21] Prasanna V, Prabha TN, Tharanathan RN. Fruit ripening phenomena—An overview. *Critical*  
377 *Reviews in Food Science and Nutrition*. 2007; 47: 1–19. DOI:  
378 10.1080/10408390600976841.

379 [22] Babalar M, Asghari M, Talaei A, Khosroshahi A. Effect of pre- and postharvest salicylic acid  
380 treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit.  
381 *Food Chemistry*. 2007; 105: 449–453. DOI: 10.1016/j.foodchem.2007.03.021.

382 [23] Wang P, Sun X, Xie Y, Li M, Chen W, Zhang S, Liang D, Ma F. Melatonin regulates proteomic  
383 changes during leaf senescence in *Malus huprehensis*. *Journal of Pineal Research*. 2014; 57:  
384 291–307. DOI: 10.1111/jpi.12169.

385 [24] Forney CF, Kalt W, Jordan MA. The Composition of Strawberry Aroma Is Influenced by  
386 Cultivar, Maturity, and Storage. *HortScience*. 2000; 35: 1022-1026.

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395 TABLES

396 Table 1 Relative abundance of two VOCs that were significantly affected by the melatonin treatment

VOC	Ratio of relative abundance between mealotinin treated and untreated fruit ( $\pm$ SD)*
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	Day 0		Day 4	
	control	+MT	control	+MT
Butanoic acid, 2-methyl-, ethyl ester	0.362 $\pm$ 0.05 <sup>a</sup>	0 <sup>b</sup>	0.03 $\pm$ 0.06 <sup>a</sup>	0.02 $\pm$ 0.04 <sup>a</sup>
Hexanoic acid, ethyl ester	17.3 $\pm$ 2.38 <sup>a</sup>	21.9 $\pm$ 4.99 <sup>a</sup>	5.94 $\pm$ 1.38 <sup>a</sup>	2.80 $\pm$ 0.74 <sup>b</sup>

\*different letters indicate significant differences ( $P < 0.05$ ) based on a student t-test between treated and untreated samples

## FIGURE LEGENDS

**Fig 1:** (A) Weight loss, (B) decay, and (C) TSS of strawberry fruits treated postharvest with 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$  °C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).

**Fig 2:** (A) Titratable acidity, (B) pH, and (C) Anthocyanin of strawberry fruits treated postharvest with 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$  °C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).

**Fig 3:** (A) CO<sub>2</sub>, (B) total phenol, and (C) taste index of strawberry fruits treated postharvest with 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$  °C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).

**Fig. 4.** Canonical Analysis of Principal ordinates related to time of storage based on all 50 strawberry VOCs using TD-GC-TOF-MS: Each ellipse represents the 95% confidence interval. The plots use

421 linear discriminants LD1 and LD2 with a percentage of correct classification of 100% and  $P < 0.001$   
422 (n=6).

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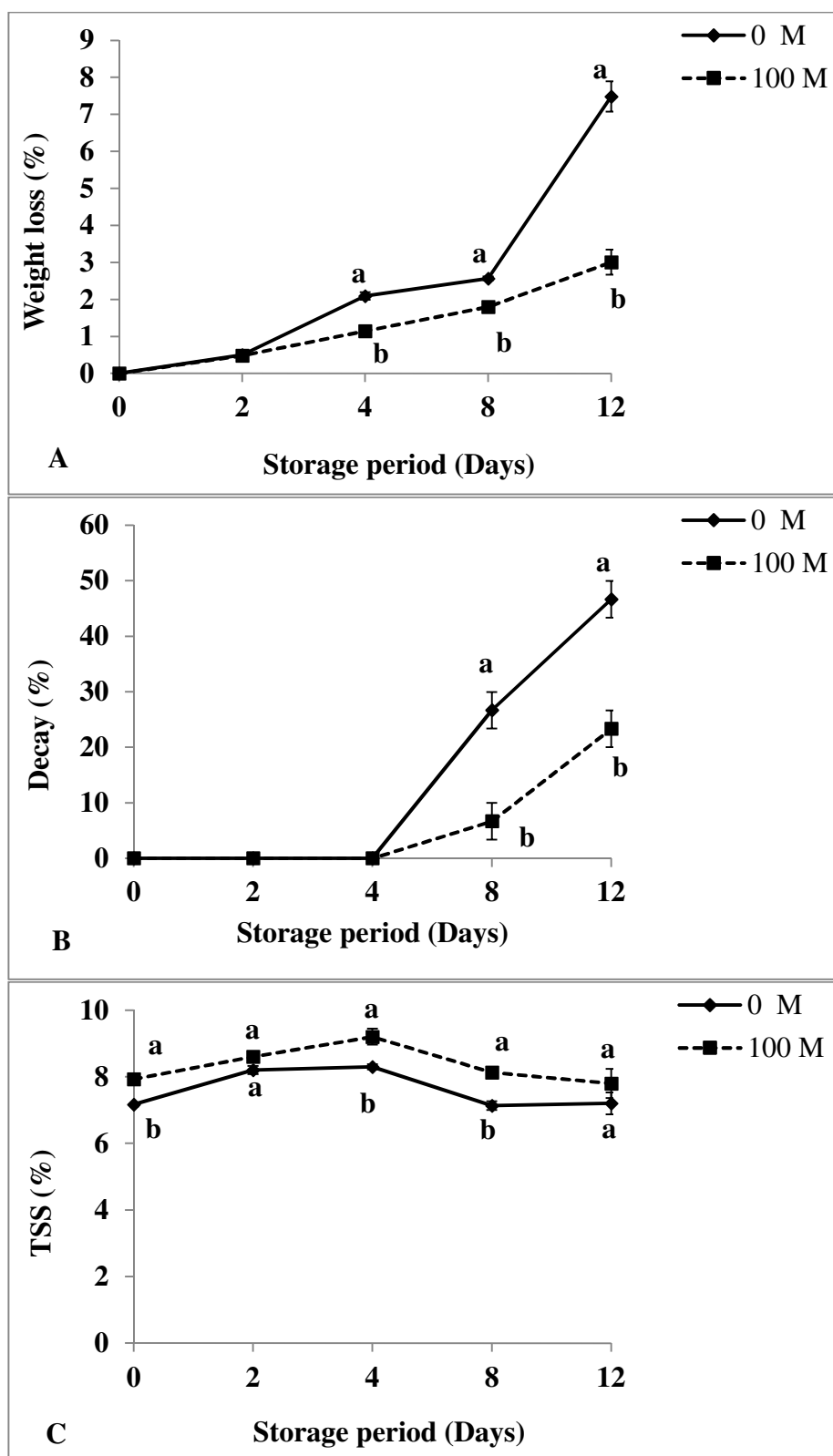
## 425 **SUPPLEMENTARY TABLES**

426 **Supplementary Table 1:** Statistical analysis of effect of storage duration on physical and chemical  
427 parameters.

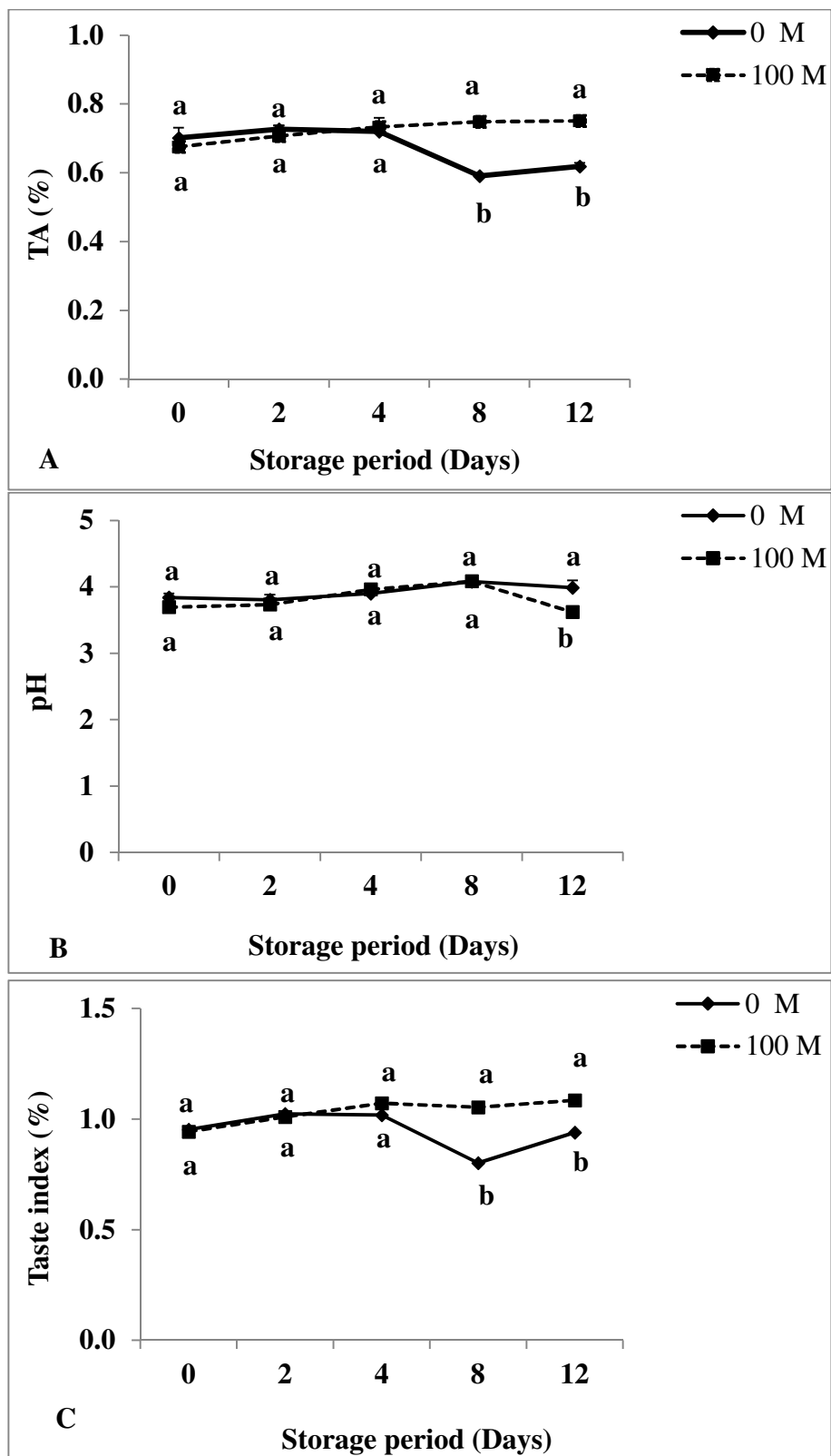
428 **Supplementary Table 2** - Emission of ethylene ( $\mu\text{l kg}^{-1} \text{ h}^{-1}$ ) from strawberry fruit treated with  
429 melatonin and controls.

430 **Supplementary Table 3** - Volatile organic compounds detected in all strawberry fruit samples

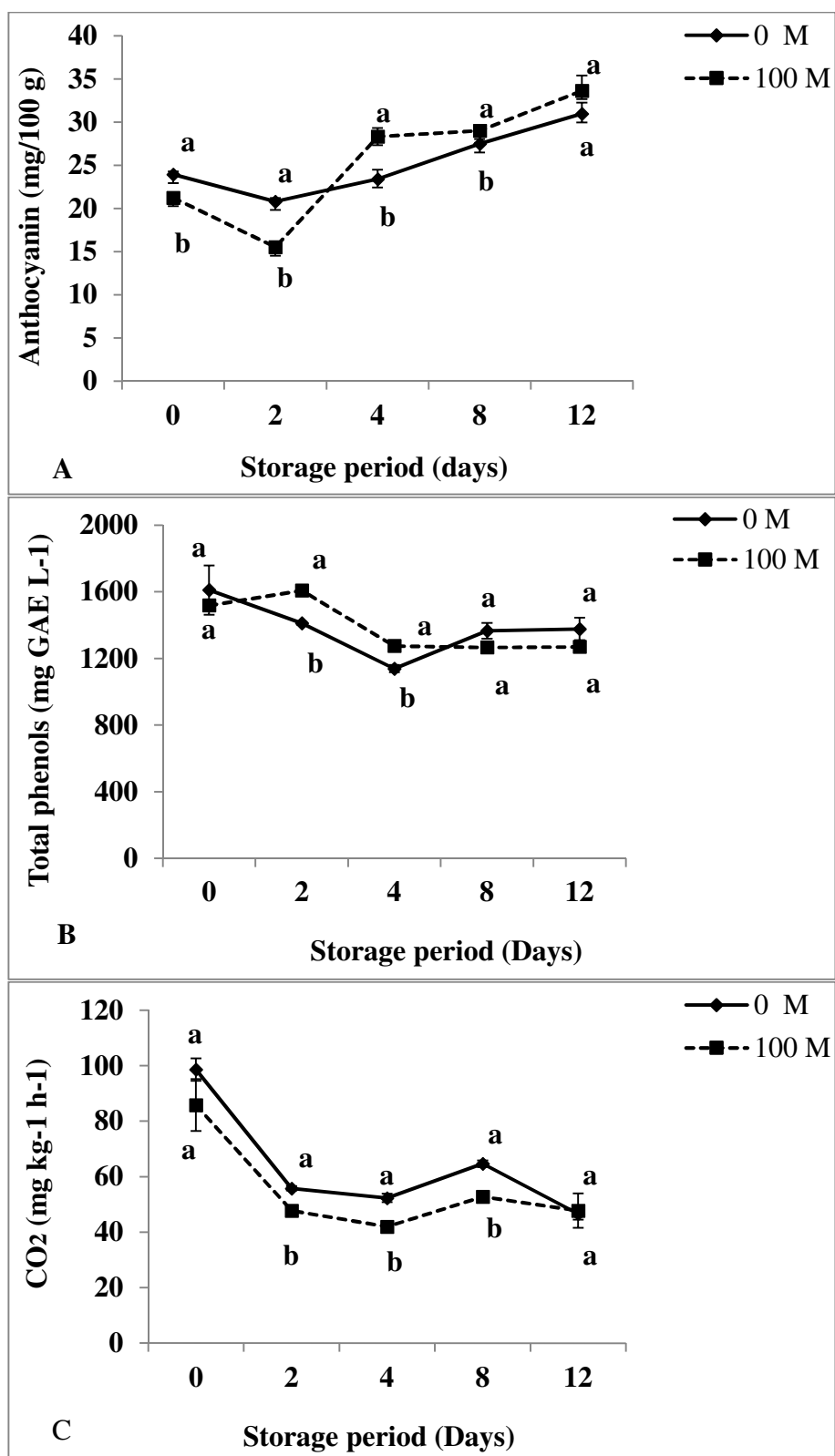
431 **Supplementary Table 4** - % abundance of each VOC across 3 replicates of each treatment at each  
432 time point including fold change and significance of change.



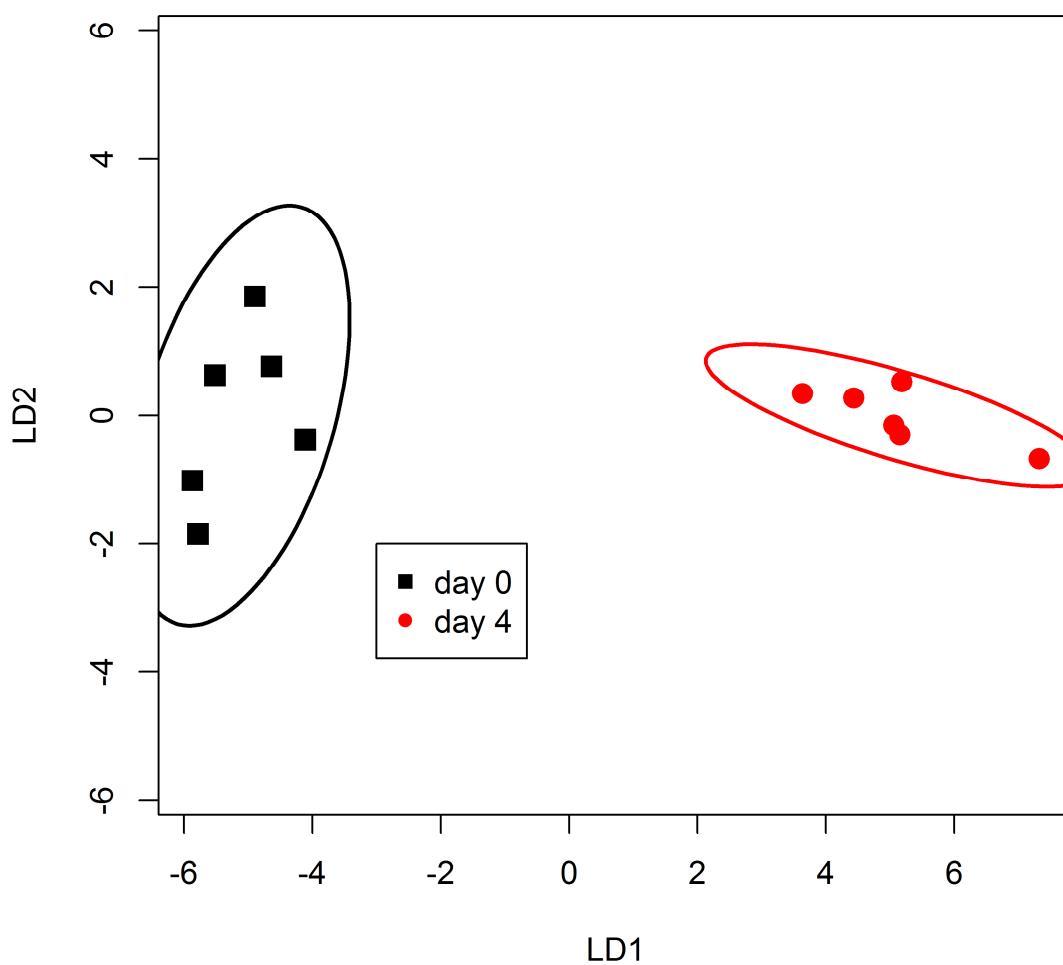
**Fig 1:** (A) Weight loss, (B) decay, and (C) TSS of strawberry fruits treated with postharvest 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$  °C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).



**Fig 2:** (A) Titrable acidity, (B) pH, and (C) taste index of strawberry fruits treated with postharvest 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$   $^{\circ}$ C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).



**Fig 3:** (A) Anthocyanin, (B) total phenols, and (C) CO<sub>2</sub> of strawberry fruits treated with postharvest 0 and 100  $\mu$ M melatonin and stored at  $4 \pm 0.5$  °C for up to 12 days. Data shown are mean values of  $n = 3$  and the error bars represent standard errors of the means. Different letters indicate significant differences between melatonin treated and control fruit at each time point (Tukey test at  $P < 0.05$ ).



**Fig. 4.** Canonical Analysis of Principal coordinates related to time of storage based on all strawberry VOCs using TD-GC-TOF-MS: Each ellipse represents the 95% confidence interval. The plots use LD1 and LD2 with a percentage of correct classification of 100% and  $P < 0.001$  ( $n=6$ ).